



*Original Contribution*

**REACTION OF THE PITUITARY-ADRENAL GLANDS CHAIN  
DURING ADAPTATION IN INDUSTRIAL PIG BREEDING**

**P. Petkov<sup>1</sup>, R. Kecheva<sup>2</sup>, D. Kanakov<sup>1</sup>**

<sup>1</sup>Department of Internal Diseases; <sup>2</sup>Department of Animal Husbandry,  
Section Radiobiology and Radioecology  
Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

**ABSTRACT**

The expression of many inducible genes involved in cell growth and differentiation as cytokine genes is regulated mainly by mitogen-activated protein kinases (MAPK)-signaling pathways. In this study we examined how JNK(SP600125) and p38(SB202190) MAPKs inhibitors influenced the inducible IL-12p40, IL-12p70 and IL-23 production. The quantity determination of these cytokines was performed by ELISA in culture supernatants. The inhibition of both JNK and p38 increased IL-12p40 production induced by all stimuli. The inhibition of p38MAPK downregulated IL-23 production and upregulated IL-12p40/p70 production in stimulated cells. We suggest the benefit of p38 control in the treatment of inflammatory and/or autoimmune diseases.

**Key words:** IL-12p40, IL-12p70, IL-23, JNK, p38.

**INTRODUCTION**

The specialization and concentration of the different branches of livestock production and the conversion of rearing systems to an industrial scale on the background of management and technical requirements, are leading to the emergence of new challenges. In particular, due to unknown reasons, the industrial technologies do not allow the full utilization of the potential of organisms and also are causing the appearance of a number of pathologies that are rarely encountered in other conditions.

Despite the considerable reduction of losses due to infectious and parasitic disease, the proportion of costs related to non-infectious diseases became especially high – 96–98% of morbidity rate records.

One of the main reasons for the non-infectious pathology are the factors of industrial technologies that are stressful for organisms (1, 2, 3, 4, 5).

Under the contemporary conditions, stress could occur during transportation (import or translocation of livestock farms) and it included both physical and emotional (social stress) components (6, 7, 8).

The patterns of the technologies of production, because of the complex influence of altered conditions, results in reduction of the activity of both specific and non-specific resistance, alteration in the content of corticosteroids, increased susceptibility to various non-infectious disorders, primarily related to the mineral metabolism (9, 10, 11).

The investigation of the functional state of the internal secretion glands and in particular, the pituitary-adrenal system under the effect of various technological factors, allowed us to assume that they influence in different manner one or another endocrine gland.

There is also no solution to the question whether the adaptive systemic reaction could occur with all bioconstants remaining within the physiological range, without fulfilment of stress reaction stages.

All these issues necessitate additional studies in order to elucidate the role of the pituitary-adrenal chain in the present organization conditions in industrial pig breeding in Bulgaria.

The locomotor activity of animals is also very important in industrial pig breeding. In conditions of hypokinesia the static loading experienced by the animals could be excessive. Despite many other stress factors, the continuous limitation of the locomotor activity results in changes described as

\* **Correspondence to:** *Petko Petkov, Department of Internal Diseases, Faculty of Veterinary Medicine, Stara Zagora 6000, Bulgaria; Tel.:+359-42-699 525*

chronic stress.

The data about the effect of hypodynamia in the hormonal activity of pituitary and adrenal glands are especially interesting.

Aside increased corticosteroid secretion, hypodynamia causes also other alterations related to increased ACTH concentrations, increased mass of adrenals that could finally result in depletion of the pituitary-adrenal system (12, 13, 14).

The requirements of contemporary technologies and the related transportation are often associated with development of acute stress. It is determined by multiple factors, the most important of them being the psychic, physical and vestibular load, temperature factors, and impairment of the usual circadian rhythms. They all induce alterations in the hormonal activity of pituitary and adrenal glands – a fact, confirmed by blood concentrations and the excretion of metabolites with urine.

The changes in the diet of the different groups of pigs could also provoke a systemic reaction, close to stress ones.

Together with mentioned factors connected with industrial technologies, pigs experience also other types of stress: moving into unfamiliar boxes, formation of groups, overcrowding, insufficient nutrition front, early weaning, noise, etc.

This variety of factors influencing the processes of adaptation in industrially reared pigs does not specify the leading role of individual factors in the process.

Without elucidation of the principal role of the pituitary-adrenal system in adaptation, the reaction of the chain in conditions of transportation stress (introduction) remains unclear as well as the influence from the part of changed environment, the technological allotment into groups, physical condition etc.

The purpose of the present study was to make clear the extent of the physiological tension of the system in the real management conditions of industrial pig breeding in Bulgaria with regard to the possibilities for control and for realization of the genetically determined potential in the main introduced breeds, lines and their crosses.

## MATERIAL AND METHODS

The investigations were carried out in the following commercial pig holdings: “Hibriden center po svinevadstvo” (HCS) Ltd, Shoumen and “Hybrid Centre Invest” (HC) Ltd, village of Radko Dimitriev, municipality of

Shoumen. In the investigation, 95 pigs, divided in 4 groups according to introduction variants, were included:

- First group – HCS Shoumen; according to the age – three, four and five month-old female Danish Yorkshire pigs and four-month-old male Danish Landrace pigs.
- Second group – HCS Shoumen – according to breed and sex: Danish Duroc /DD ♀/, Danish Duroc /DD ♂/, Danish Landrace /DL ♀/, Danish Landrace /DL ♂/, Danish Yorkshire /DY ♀/, Danish Yorkshire /DY ♂/.
- Third group – HCS Shoumen; according to the physiological condition: Danish Yorkshire replacement females /R ♀/, pregnant-first half of pregnancy /Pr.Ist ½/, pregnant- second half of pregnancy / Pr. IInd ½/, lactating sows, dry sows.
- Fourth group – HC- Radko Dimitriev-according to the breed and sex: Big White female pigs /BW ♀/, Landrace /L ♂/, Big White ♀× Landrace ♂ crosses /F<sub>1</sub>-♀/ and English Duroc males /ED ♂/.

The breeding system was free, in pens. The animals from both centres were placed under the same conditions of feeding, immunoprophylaxis and antiparasitic treatment. The blood samples were obtained from sinus ophthalmicus.

1. ACTH quantitation. The determination of plasma ACTH was done in EDTA blood plasma with RIA-mat ACTH kit produced by Mallinckrodt Diagnostica with the following characteristics:

- species specificity – the ACTH antibody is specific for the assayed porcine ACTH. The cross-reactivity percentage calculated according to Abraham showed 100% specificity against the used antiserum.
- sensitivity – the least detectable ACTH amount with this test was 20 pg/ml /15, 16/.

2. Cortisol quantitation. Cortisol was quantitated in blood serum or heparin, EDTA plasma by means of Cortisol RIA-Coated Tube Radioimmunoassay kit (Orion Diagnostica, Finland) with the following characteristics:

- species specificity – the cortisol antiserum binds 100% of cortisol contained in the sample, 6.5% of corticosterone and under 1.0% of cortisone, deoxycortisone and aldosterone.

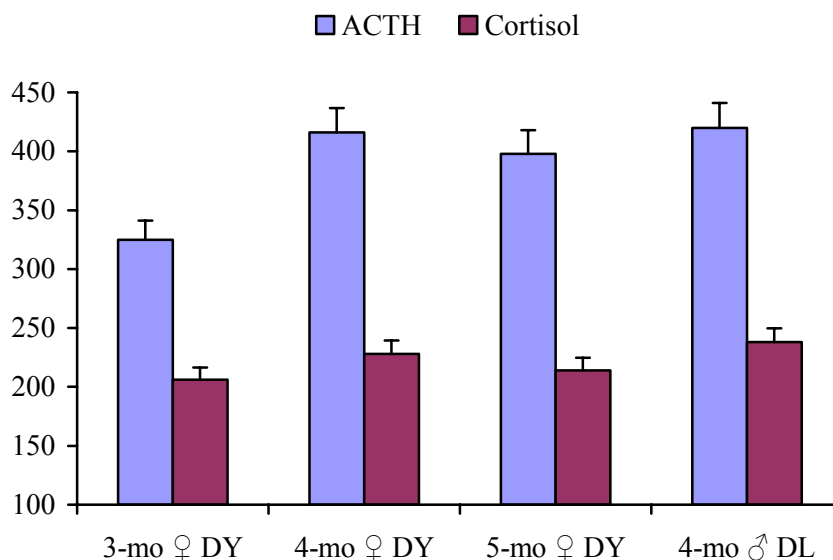
- sensitivity – the least detectable cortisol concentration with this test was 5 nmol/l /17, 18/.

The results of per each group were analysed, the average values, the standard deviations and the standard errors of means being calculated. The Student's t-test was used to determine the statistical significance between means in the different groups and between the

different ages.

## RESULTS AND DISCUSSION

The data about the changes in ACTH and cortisol concentrations due to the long-time transportation – introduction and the age when this occurred, are shown on **Figure 1** and **Table 1**.



**Figure 1.** ACTH and cortisol concentrations during the process of introduction of pigs.

**Table 1.** ACTH and cortisol concentrations during the process of introduction of pigs.

Parameter	3-mo ♀ DY	4-mo ♀ DY	5-mo ♀ DY	4-mo ♂ DL
ACTH	325±14	416±17	398±16	420 ±17718
Cortisol	206±9	228±11	214±10	238±12

The reaction of the pituitary gland, evaluated via ACTH levels showed that the introduction of pigs at the age of 3, 4 or 5 months, regardless of the sex, resulted in pituitary activation manifested by significant elevation of ACTH in 4- and 5-month-old pigs.

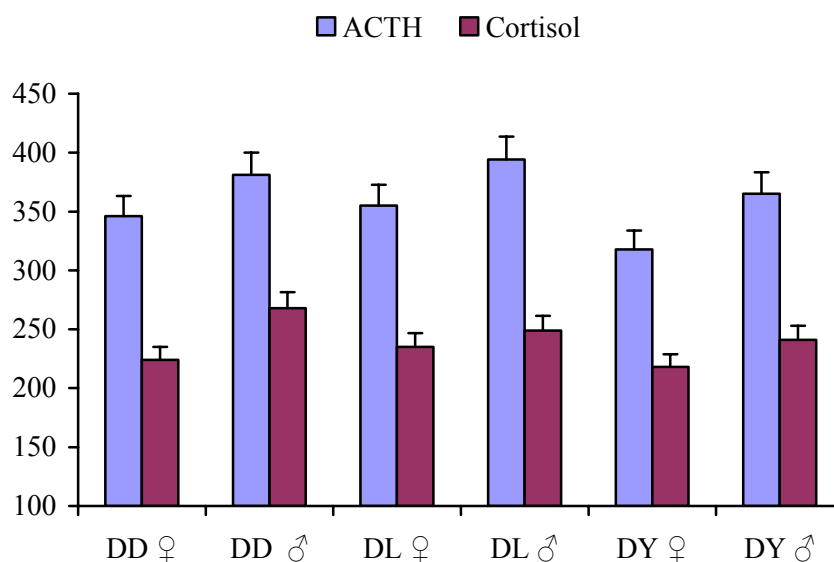
Thus, in pigs introduced at the age of 3 months ACTH blood concentrations were 325±14 pg/ml, and in 4- and 5-month-old pigs these values were significantly higher and reached 416±17 pg/ml and 398±16 pg/ml, respectively.

At the same time, adrenal glands did not react to the increased ACTH level and although a tendency towards higher cortisol levels were observed, it remained close to the upper physiological norm and for the different age groups, ranged between 206±9 and 238±12 nmol/l.

The results for the influence of the

breed and sex on ACTH and cortisol levels are shown on **Figure 2** and **Table 2**.

Having detected the effect of breed and sex, it was found out that the representatives of the Danish Duroc and Danish Landrace had a rather considerable adaptation potential and that the pituitary reaction compensated for the technology-induced factors. ACTH in these two breeds ranged between 346±13 and 394±14 pg/ml regardless of the sex. The Danish Yorkshire pigs were susceptible to the alterations in technological conditions. In them, though a certain sex-related differentiation was observed, ACTH levels were from 318±12 to 365 ±14 pg/ml and lower compared to Danish Duroc and Danish Landrace pigs, whereas in male pigs, they were higher compared to Duroc and Landrace females.



**Figure 2.** ACTH and cortisol concentrations depending on the breed and sex

**Table 2.** ACTH and cortisol concentrations depending on the breed and sex

Parameter	DD ♀	DD ♂	DL ♀	DL ♂	DY ♀	DY ♂
ACTH	346±12	381±14	355±13	394±14	318±12	365±12
Cortisol	224±8	268±10	235±9	249±10	218±8	241±9

Comparing the pituitary reaction and that of adrenals under similar conditions, it could be established that the reaction of the latter followed the pattern of positive and negative feedbacks.

Accounting for the interaction in the current conditions on the background of breed- and sex-related peculiarities, cortisol concentrations were between 218±8 and 268±10 nmol/l. The analysis of data demonstrated that significant increase was present in male Danish Duroc not only against female pigs from the same breed, but also against all other breed and sex groups. In spite of the more dynamic reaction of the pituitary gland, adrenals either did not react or reacted very slightly.

The results of the combined effect of the technological arrangement of the complex and the physiological state of pigs upon the pituitary-adrenal system are presented on **Figure 3** and **Table 3**.

The analysis of ACTH levels showed that in the group, they varied between 331±12 and 396±14 pg/ml. Despite the broad range of variations, pregnant sows in their 1<sup>st</sup> and 2<sup>nd</sup> trimester of gestation exhibited significant difference in favour of the sows at the 2<sup>nd</sup> trimester with higher values than those at the 1<sup>st</sup> trimester (396±14 pg/ml vs 331±12 pg/ml, respectively). The values of these parameters

in the groups formed on the basis of the physiological state showed that pregnancy was a leading factor in the pituitary reaction. The observed changes in ACTH levels associated with the physiological state were also confirmed by cortisol concentrations.

From 248±9 nmol/l in replacement pigs, cortisol concentrations sharply decreased during the 1<sup>st</sup> trimester of gestation to 204±8 nmol/l. During the 2<sup>nd</sup> trimester, cortisol varied about 282±10 nmol/l and that was significantly higher compared to both sows in the 1<sup>st</sup> trimester and dry sows.

The resulting blood cortisol concentrations demonstrated that they could be centrally (hypothalamus-pituitary) or peripherally induced (ovaries-placenta).

The level of activity of the pituitary-adrenal chain in the Big White, Landrace breeds and their F<sub>1</sub> crosses are presented on **Figure 4** and **Table 4**.

According to our data, the F<sub>1</sub> crosses (BW ♀ x L♂) reacted less intensely or exhibited a higher potential for adaptation compared to the source parental line (BW ♀). The analysis of this parameter showed an adjustment of adaptive mechanisms that justify to a significant extent the approved reproduction pattern. ACTH values in male Landrace of 432±14 pg/ml were higher than in female Big White sows and the first

generation F<sub>1</sub> and at the same time, higher compared to the next member in the process

of reproduction: English Duroc ♂ (391±13 pg/ml)

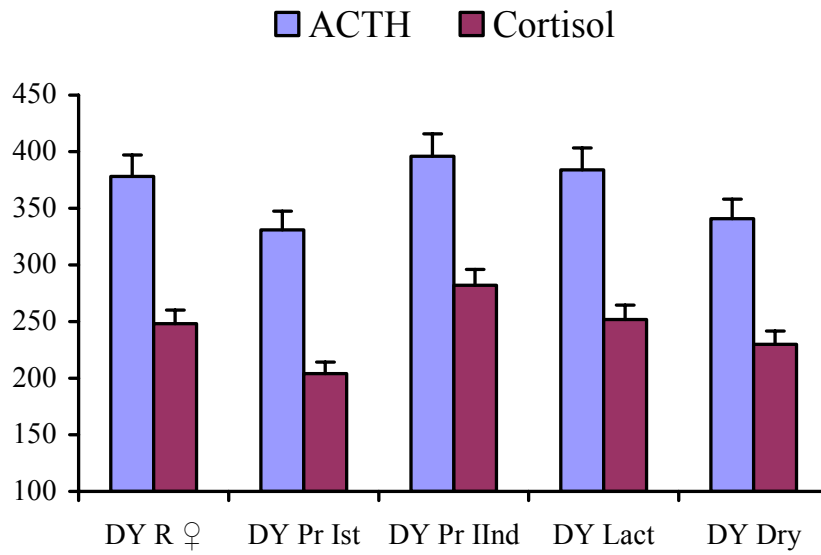


Figure 3. ACTH and cortisol concentrations depending on the physiological state

Table 3. ACTH and cortisol concentrations depending on the physiological state

Parameter	DY R ♀	DY Pr Ist	DY Pr IInd	DY Lact	DY Dry
ACTH	378±14	331±12	396±14	384±13	341±12
Cortisol	248±9	204±8	282±10	252±9	230±8

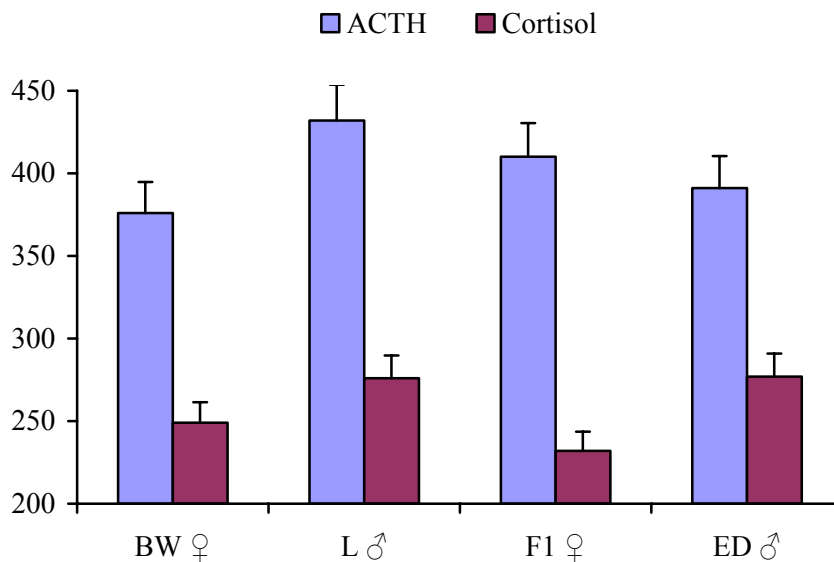


Figure 4. ACTH and cortisol concentrations depending on the breed

Table 4. ACTH and cortisol concentrations depending on the breed

Parameter	BW ♀	L ♂	F1 ♀	ED ♂
ACTH	376±13	432±14	410±12	391±13
Cortisol	249±9	276±9	232±8	277±9

The accepted reproduction schedule also was found to stabilize the adrenal reactions.

The reaction of adaptation manifested by the variations in blood cortisol concentration showed that baseline values of  $249 \pm 9$  nmol/l in female Big White, were significantly lower than those in male Landrace ( $276 \pm 9$  nmol/l), whereas their  $F_1$  crosses were with average cortisol levels of  $232 \pm 8$  nmol/l, close to cortisol levels of the maternal line. These values were lower than those determined in male English Duroc boars ( $277 \pm 9$  nmol/l) that are included in the reproduction of  $F_2$ .

## CONCLUSION

The results from the present investigation on the reaction of the pituitary-adrenal chain showed that the studied groups preserved the functional relationships of pituitary and adrenal glands and thus, allowed an active adaptation in conditions of altered technological parameters.

According to our data, the groups formed according to technological traits and physiological conditions, the pituitary-adrenal chain had a primary role in the development of the so-called adaptive stress.

The analysis of the data from the first series of experiments showed that the long-term transportation in the course of introduction of new pig breeds and lines was related to changed geographical and climatic conditions and thus, was a non-specific stress factor manifested by an early increase in both controlled parameters: ACTH and cortisol.

Our data, confirmed by the studies of Sudakov (19), showed that the reaction of both systems occurred according to the dominance principle.

ACTH and cortisol concentrations in groups formed on the basis of breed and gender, showed that they had a genetically higher potential for adaptation expressed through a higher stability of studied parameters.

The effect of other factors on ACTH and cortisol concentrations, established in the groups with different physiological condition, breed, reproduction schedules allowed us to assume that the animals from these groups were able to compensate for the external influence, the intensity and the duration of challenging factors by mobilization of pituitary-adrenal system, but the changes in ACTH and cortisol corresponded to the physiological norms.

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